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ENDOTOXIN-PROTECTION OF MICE. THE RELATIONSHIP TO COLONY-FORMING UNITS

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ABSTRACT

Earlier studies have shown that bacterial endotoxins increase survival of irradiated animals. Although these substances do not confer as much protection as do the classical chemical protectants, endotoxins do significantly increase survival when given either before or for a short time following irradiation. The mechanisms of endotoxin protection have not been clearly established, but earlier studies have shown that hematopoietic stimulation is involved. The present studies were designed to evaluate the effects of endotoxin on proliferative cells in the hematopoietic system. Methods have recently been devised to measure the numbers of certain proliferative cells within the bone marrow, spleen, and other hematopoietic sites. These proliferative cells are called colony-forming units (CFU's), and their numbers are estimated by counting the "colonies" or nodules in the spleens of irradiated mice which arise either "spontaneously" or after injection of hematopoietic cells. The present data indicate that endotoxin does not alter the radiosensitivity of endogenous splenic CFU's; the D_{37} for CFU's is ~ 90 R in both endotoxin-treated and control mice. When endotoxin is injected at the time which produces optimal survival, the femur content of CFU's increases ~ 2 fold; whereas, the spleen content of

CFU's is increased ~ 20 fold. The increased rate of CFU's migration from the femur to the spleen in endotoxin-treated animals may contribute to the relatively greater increase in splenic CFU's. The diameter of the CFU's in the endotoxin-treated mice is somewhat larger than in controls which may have implications relating to rates of cell division. Other data are presented which relate to the extent to which time of endotoxin injection and numbers of splenic CFU's can be correlated with survival of irradiated mice.

SUMMARY

The Problem:

Hematopoietic stimulation probably plays an important role in the mechanism of endotoxin-protection of irradiated animals. Methods have recently been devised by which certain hematopoietic cells can be enumerated by counting colony-forming units (CFU's) or spleen nodules in irradiated mice. The purpose of the present study was to determine if the number of CFU's, which may be hematopoietic stem cells, could be correlated with the extent of endotoxin-protection.

The Findings:

Since the degree of endotoxin-protection is influenced by time of injection relative to irradiation, a time-course study was conducted with endogenous spleen CFU's to determine the extent to which changes in numbers of CFU's could be correlated with the survival of mice. The time relationships are similar only in that the maximum number of CFU's is observed when maximal survival is observed; that is, when endotoxin is injected 24 hours before irradiation. At other times of endotoxin injection, the numbers of CFU's do not predict the degree of protection. The D_{37} for splenic CFU's is similar, ~ 90 R, in endotoxin-treated and control mice; endotoxin, therefore, does not alter the radiosensitivity *per se* of CFU's at the time of maximal endotoxin-

protection. Endotoxin increases the content of femur CFU's by a factor of ~ 2, and the migration rate of CFU's from a femur to spleen is increased for several hours after endotoxin injection. Also the splenic content of CFU's is increased by a factor of ~ 20 and the size of the nodules tends to be larger in endotoxin-treated mice. The extent to which CFU's may be related to endotoxin-protection is discussed.

INTRODUCTION

Among the effects of bacterial endotoxins is that of protection against the effects of whole-body irradiation in mice, rats, hamsters and dogs (1-7). This radio-protective effect has been demonstrated by an increase in the LD_{50}; by increased survival of endotoxin-treated mice following a fixed radiation dose; and by an accelerated recovery of peripheral blood cells in endotoxin-treated animals following a sub-lethal whole-body exposure.

The timing of the endotoxin injection relative to the time of irradiation is important. The maximal protective effect is usually obtained when the endotoxin is administered approximately 24 hours before irradiation, although some protection is observed when the endotoxin is given up to several days before irradiation or shortly after midlethal exposure. At minimal effective doses the intravenous route of administration has been found to produce the greatest amount of protection; repeated injections have been shown to abolish or markedly diminish the radio-protective effect (1,2,7,8).

Endotoxins produce many physiological responses which may be related to the radio-protective effect. These include hematopoietic stimulation, leukocytosis, platelet sequestration perhaps with serotonin release, adrenal stimulation, reticulo-endothelial system hyperplasia,

and fever (9, 10). The relative importance of these responses to the radio-protective effect of endotoxins has not been established.

The recent development of methods for evaluating the numbers of colony-forming units (CFU's) (stem cells?) in the bone marrow or spleen of the mouse has provided a model by which the radio-protective effect of endotoxin may be studied at the level of what may be the stem cell (11, 12). The purpose of the present study was to determine the extent to which the radio-protective effect of bacterial endotoxin could be correlated with changes in numbers of CFU's in the bone marrow and spleen.

MATERIALS AND METHODS

Experimental Animals

Female LAF_1 or CF#1 mice between 3 and 4 months of age from three sources were used in these studies. Some LAF_1 mice were bred in this laboratory and others were obtained from Jackson Laboratories (LAF_1/JA). Because mice from the latter source showed greater variability in size, weight, and a slightly different response to radiation, individual experimental series were restricted to LAF_1 animals from one of the two sources. CF#1 mice were obtained from Carworth Farms.

Mice obtained from outside the laboratory were maintained in quarantine for three weeks, then placed in experimental mouse housing areas with the mice produced in the laboratory. All of the animals were housed 10 per cage before assignment to experimental groups, and were

allowed food and water ad libitum. The water was acidified with hydrochloric acid to a pH of 2.6 in an attempt to reduce cross infection.

Total Body Irradiation

Mice were put in lucite tubes which were placed on a rotating turntable and were given whole-body exposures to 250 kvp X rays. The TSD was 40 in., HVL 1.49 mm. Cu and dose rate 28-30 R/min. Dosimetry with abdominal implants of lithium floride capsules indicated a roentgen to rad conversion factor of ~ 1.2 for this physical arrangement.

Endotoxin

The endotoxin used in these experiments was PIROMEN, a highly purified lipopolysaccharide derived from Pseudomonas and kindly supplied by Flint Eaton and Company in concentrations of 1000 µg/ml. The usual dose in these experiments was 50 µg/mouse injected intravenously in 0.05 cc. volume; this dose produced no signs of acute toxicity in either strain of mouse.

Hematology Studies

Hematologic studies were performed on blood obtained from the tail by threading a 20 gauge needle a short distance up a tail vein. Mice bled in this manner may be bled daily for over two weeks without developing signs of local infection or anemia. This method allows enough blood to be obtained for routine total and differential white blood cell counts.

Endogenous Spleen Nodule Technique

As shown by McCulloch and Till, mice given moderate exposures to whole-body radiation have regenerating nodules in their spleens when examined several days later (11). Individual groups of 10-20 mice were given a whole-body exposure, and transferred to single cages where they were maintained on Purina food and water containing 100 mgm% of neomycin and 840 units/ml. of polymixin. Eight days after irradiation, the mice were sacrificed and the number of spleen colonies was determined by counting with the aid of a dissecting microscope; all spleen nodules with a diameter of .25 mm. or larger were counted.

Bone Marrow Migration Technique

Previous studies have shown that cells capable of forming spleen colonies are released at a constant rate from shielded bone marrow sites into the circulation (13,14). LAF_1/JA female mice were anesthetized with nembutal, shielded with 2 mm. of lead posterior from the last row of nipples, and given 900 R partial body exposure (250 kvp, 15 MA, 35 cm TSD, 100 R/min.). One hour after the conclusion of the first exposure, the area previously shielded was given a 900 R exposure to eliminate the source of migratory colony-forming units (CFU's). The animals were than caged singly, and 8 days after irradiation they were sacrificed and spleen colonies were counted. Colonies formed in the spleens of these animals resulted from cells that had migrated from the shielded femur during the one hour interval between irradiations. With this

technique the numbers of CFU's migrating to the spleen in a one hour period was determined at various times after an intravenous injection of endotoxin.

Experimental Design for Determining Colony-Forming Units Content of the Femur

Bone marrow was obtained from the femurs of control mice and from mice given endotoxin 24 hours before sacrifice by removing the femoral head, drilling a hole in the distal end with a 25 gauge needle and flushing the marrow out with sterile Hanks solution. Cells obtained from 2-4 femurs were pooled, dispersed, and nucleated cells counted in a hemocytometer. Appropriate dilutions were then made, and the cells were injected into lethally irradiated recipients to determine colony-forming units (CFU's) per 10^5 cells. From these data it was possible to calculate the mean numbers of nucleated cells per femur, the number of CFU's per 10^5 nucleated cells, and total number of CFU's per femur.

RESULTS

Effect of Route of Administration of Endotoxin

Figure 1 shows the comparative effect of various routes of administration of 50 μg of endotoxin on the numbers of endogenous colony-forming units (CFU's) counted in spleens eight days after 700 R whole-body irradiation. The mean number of CFU's is plotted, and the bar indicates ± 2 standard errors of the mean. The intravenous route

of injection produced twice as many nodules as the subcutaneous, and about 40% more than the intraperitoneal route.

Effect of Endotoxin Dose

Table 1 shows the effect of endotoxin dose on the number of endogenous spleen colony-forming units (CFU's) counted eight days after 630 R whole-body irradiation. Endotoxin was administered intravenously 24 hours before irradiation. The greatest number of CFU's was seen after 25 μg, although in this experiment the number of nodules per spleen was too high for accurate counting. The response produced by 50 μg indicated that the optimum dose had been exceeded as reflected by a modest reduction in the number of CFU's. Untreated mice showed less than one CFU's per spleen after 630 R whole-body irradiation.

The Effect of Multiple Injections of Endotoxin

Table 2 shows the effect of multiple injections of endotoxin on the number of endogenous spleen nodules counted eight days after a 700 R whole-body exposure in LAF_1/JA mice. Repeated intravenous injections of 50 μg, 8 or 14 days before a second injection given 24 hours before irradiation resulted in fewer colonies than did a single 50 μg injection.

Effect of Endotoxin on $LD_{50/30}$

Table 3 shows the LD_{50}'s for non-injected controls and for LAF_1 mice injected intravenously with 50 μg of endotoxin 24 hours before irradiation. Endotoxin increased the $LD_{50/30}$ by 217 R. The slopes of the exposure-response curves did not differ significantly.

Hematologic Response to Intravenous Endotoxin

Figure 2 shows the changes in the numbers of peripheral leukocytes over a four day period after the intravenous injection of 50 μg of endotoxin. Maximum lymphocyte depression was evident between 4 and 12 hours after injection, and this was followed by a return to normal by 30 hours. Polymorphonuclear leukocytes were rapidly mobilized, reaching a peak of eight times normal 8 hours after injection, and by 24 hours were within the normal range. The 95% confidence limits are plotted for each mean.

Total Content of Femur Colony-Forming Units

The data used in determining total femur colony-forming units (CFU's) content are summarized in Table 4. Three separate determinations of femoral CFU's were made 24 hours after endotoxin administration in non-irradiated LAF_1 mice. The mean values were 1.44×10^7 nucleated cells per femur, 56.2 CFU's per 10^5 cells and 8093 total CFU's per femur. Mean values from four determinations in control animals utilizing a total of 14 femurs were 1.53×10^7 nucleated cells per femur, 31.1 CFU's per 10^5 cells and 4750 total CFU's per femur. These data indicated a 1.70-fold increase in total femur CFU's content 24 hours after endotoxin administration.

Effect of Endotoxin on Colony-Forming Units Migration to the Spleen

Figure 3 shows that endotoxin produced a transient increase in the rate of migration of colony-forming units from the bone marrow to the

spleen. The migration rate was approximately doubled at 4 and 8 hours after the administration of endotoxin. This was followed by a rapid return to normal and a suggestion of a second damped cycle. The means are based on 18 to 23 spleens per point, and the bar includes ± 2 standard errors. There was unusually high mortality in the individual experimental groups (40-50%) because of the necessity of double exposure (1800 R) to a portion of the bowel.

The Effect of Time of Injection of Endotoxin on Endogenous Spleen Colony-Forming Units

The effect of time of injection of endotoxin relative to the time of irradiation is shown for CF#1 mice in Figure 4A (600 R total body exposure) and Figure 4B (700 R total body exposure). Similar studies in IAF_1 female mice are shown in Figure 4C (550 R total body exposure) and Figure 4D (700 R total body exposure). The number of nodules reached a maximum when the endotoxin was given 24 hours before irradiation. A sharp decline followed, and the effect was essentially absent when the endotoxin was given 24 hours after the irradiation. The means plotted with asterisks are estimates, because nodules in those spleens were too numerous for accurate counting. These estimates were made by doubling the minimum count in the group. The remaining means and the control ranges include ± 2 standard errors.

Based on animals exposed to 700 R, in which the nodules were readily

countable, the peak colony-forming units values indicated a minimum increase of ~ 20-fold.

The Effect of Time of Endotoxin Administration on Survival

Figure 5 is taken from earlier data obtained in BUB mice injected intravenously with Proteus morganii endotoxin at various times before or after irradiation (3). This figure is included for comparison with Figures 4A, B, C, and D. Alteration in survival of these mice when subjected to a fixed radiation exposure of 600 R at various times before and after endotoxin administration follows the same general pattern as that seen with spleen nodules.

Radiosensitivity of Endogenous Spleen Colony-Forming Units After Intravenous Endotoxin Administration

Figure 6 shows survival curves for spleen colony-forming units (CFU's) in control mice, and in mice irradiated 24 hours after an intravenous injection of 50 μg of endotoxin. The bar about each point includes ± 2 standard errors of the mean number of colonies counted in mice given graded radiation exposures. The D_{37}'s calculated from a least squares regression analysis are 87 R (78 R - 99 R) for the controls, and 94 R (88 R - 102 R) for the endotoxin-treated mice. The slopes of the two plots are not significantly different; however, the endotoxin plot is shifted on the X axis by 290 R. By extrapolating each survival curve back to the Y axis, an estimate can be made of the total CFU's content of the spleen at zero dose. These extrapolations

indicate that the control spleens contained about 2000 CFU's, while 24 hours after endotoxin the spleen contained about 28,000 CFU's. Note that the X axis in this figure begins at 300 R.

The Effect of Endotoxin on the Size of Regenerating Spleen Nodules

Table 5 shows a comparison of the mean diameters of endogenous nodules in the spleens of control animals and in the spleens of animals treated with endotoxin 24 hours before irradiation. In most cases, the nodules in the endotoxin-treated animals had a larger mean diameter than did those in the controls. These data are positioned in the table so that a horizontal comparison of control and endotoxin groups is made from spleens that contained approximately the same total number of nodules. There is no relationship of nodule size to total numbers of nodules per spleen in these data (18 nodules per spleen maximum), or to the magnitude of the radiation dose.

Figure 7 is a histogram showing the frequency distribution of individual spleen nodule sizes in control and endotoxin-treated mice. Both distributions appear to be normal, although perhaps slightly shifted to the right. In the endotoxin-treated mice, the shift to the right is accentuated. In both groups the largest sized nodules may result from coalescence of nodules formed from two colony-forming units.

DISCUSSION

Earlier studies have shown that bacterial endotoxins increase

survival of irradiated animals (1-8). Although these substances do not confer as much protection as do the classical chemical protectants, endotoxins do significantly increase survival when given either before or a short time after midlethal irradiation (1-3). The mechanism of endotoxin protection has not been clearly established, but earlier studies have shown that some form of hematopoietic stimulation is involved (16,17). The present studies were designed to evaluate the effects of endotoxin on proliferative cells in the hematopoietic system by means of spleen colony techniques (11,12). In brief, certain hematopoietic cells have the capacity to divide and form macroscopic nodules within 8 to 11 days in the spleens of irradiated mice. Each nodule may arise from a single proliferative cell which is referred to as a colony-forming unit (CFU). Spleen nodules develop in the spleens of supralethally irradiated mice injected with appropriate numbers of viable hematopoietic cells. Countable numbers of nodules also develop "spontaneously" in the spleens of mice given comparatively high sublethal exposures. Since no tissue transplantation is involved, the latter are referred to as endogenous nodules, and they are thought to represent the number of CFU's in the spleen which survive a radiation exposure. Utilizing these techniques, we have attempted to determine the extent to which endotoxin-protection could be related to the number of CFU's found in femoral bone marrow, the spleen or the hemato-

poietic system at large. If the CFU is the hematopoietic stem cell, or is representative of changes in the number of stem cells, the degree of endotoxin protection might be correlated with changes in the numbers of CFU's.

One approach used in the present studies was to determine if several factors which predictably influence the radio-protective effects of endotoxin likewise influence the CFU population in the expected manner, that is, increase the number of endogenous spleen nodules. The factors evaluated were: (1) route of injection; (2) number of injections; (3) endotoxin dose; and (4) time of injection relative to irradiation. At minimally effective endotoxin doses, the route of injection markedly influenced the protective effect; intravenous injection producing greater survival than subcutaneous injection (8). In like fashion, the greatest numbers of endogenous spleen nodules were observed in intravenously-injected mice.

Multiple injections of endotoxin (at the same dose level) are less effective in protecting mice than is a single injection (1,8,18); however, multiple incremental doses protect rats (4). The present data show that two endotoxin injections, separated by 8 or 14 days, produced fewer spleen colonies than did a single injection. The basis for the diminution in protective effect and numbers of CFU's after more than one injection is not known.

The protective effect of endotoxin increases as the dose of endotoxin is increased over the range of 2 μg to 100 μg/mouse (8,15). A qualitatively similar effect was seen over the range of 2 μg to 25 μg in terms of numbers of spleen nodules. However, a dose of 50 μg produced fewer nodules than did 25 μg. The basis for the reduction in numbers of nodules seen after 50 μg is not known. This could be related to vascular changes or toxic effects, but a dose of 50 μg produced no signs of acute endotoxin toxicity in these mice.

Earlier studies with mice have shown that the degree of endotoxin-protection is markedly influenced by both the time of injection relative to irradiation and the radiation exposure (1-3). The general relationships are as follows: as the radiation exposure increases, the number of survivors decreases; also, as the exposure increases, the range in time of effective treatment decreases. These relationships are shown graphically in Figure 5. The present data show that the number of endogenous spleen nodules observed 8 days after irradiation was also markedly influenced by the time of endotoxin injection relative to irradiation. In both strains of mice, the maximum number of nodules was observed (or estimated at the lower exposure levels) when endotoxin was given 24 hours before irradiation. Since optimal radiation-protection is afforded by endotoxin given 24 hours before irradiation, maximal numbers of spleen nodules and optimal protection appear to

correlate. The protection curve in BUB mice, and the spleen nodule curves in LAF_1 and CF#1 mice are similar in shape; however, the spleen nodule curve does not clearly predict the increased survival produced by injection after irradiation or does it predict the difference in protection seen with injection 2 or 4 days before irradiation. Comparison of results obtained with different endotoxins in different strains of mice may be tenuous, but the influence of these factors is probably not great (1,3). In order to make better comparisons, however, we are extending the protection studies in LAF_1 mice. In any event, the present comparisons indicate the number of spleen nodules does not consistently predict the temporal relationships of endotoxin protection, but this may not be surprising in view of the complexity of the system and the multiplicity of factors which influence the numbers of spleen nodules.

Endotoxin markedly influenced the numbers of CFU's in different hematopoietic sites. The present data show that 24 hours after endotoxin injection, the total number of femoral CFU's and the CFU's per 10^5 nucleated bone marrow cells were increased by ~ 70%. This estimate of the femoral CFU's increase may be somewhat low due to the trauma associated with preparation of cell suspensions and re-injection. Based on studies with femur-shielded mice injected with endotoxin, the rate of CFU migration to the spleen showed a transitory two-fold in-

crease, and this increase preceeded the granulocytosis by a few hours. The number of spleen nodules in endotoxin-treated mice indicated approximately a 20-fold increase in the number of CFU's in that site. The different increments in femoral and splenic CFU's might indicate a differential response to endotoxin of CFU's in the marrow and in the spleen; however, the position can be taken that endotoxin produces essentially the same increment in both sites and the observed differences result from redistribution of CFU's. The factors affecting CFU's content in the spleen are discussed below.

The spleen nodule data summarized in Figure 6 show that although endotoxin increased the numbers of CFU's in the spleen, the radiosensitivity per se of the CFU's was not significantly affected. The D_{37}'s for CFU's were 87 R and 94 R, respectively, in controls and in mice treated with endotoxin 24 hours before irradiation. Extrapolation of the survival curves to the Y axis indicates approximately a 15-fold increase in spleen CFU's in endotoxin-treated animals given zero exposure. This estimate of a 15-fold increase, rather than the 20-fold increase indicated by numbers of spleen nodules, is attributable to convergence of the curves resulting from the small difference in D_{37}'s.

This 15-20-fold increase in the number of CFU's in the spleen is probably not the result of in situ proliferation of the cells which have the capacity to form spleen nodules; an increase of this magnitude would require the extremely short generation time of 5-6 hours.

The following are some of the factors which may influence the number of spleen nodules in endotoxin-treated mice:

1. The *in situ* proliferation of cells, be they in an active or inactive hematopoietic pool, which have the capacity to form spleen nodules.
2. Migration of CFU's to the spleen from the bone marrow and/or preferential sequestration of CFU's in hypertrophic spleens of endotoxin-treated animals.
3. Time-dependent variations in the ability of the spleen to sequester or support proliferation of CFU's.
4. A stimulatory effect of endotoxin on the development of spleen nodules which increases the number which may be counted at 8 days; and/or preferential differentiation in cell lines which have shorter generation-maturation times.

The relative contribution of these factors is unknown, and the order of their presentation has no implications. It is most probable, however, that multiple factors influence the numbers of "endogenous" spleen nodules in endotoxin-treated mice. This produces several problems when one attempts to relate spleen colonies or CFU's to radiosensitivity of the endotoxin-treated mouse.

Recently there has been considerable interest in this question of relating an animal's radiation response, in terms of survival or death,

to "stem cell" - survival curves (19,20). The present data may be treated in this fashion if one assumes that CFU's are either "the stem cells" or that changes in the CFU population are indicative of changes in the stem cell population. The present radiation-protection data show that the $LD_{50/30}$ for treated animals is 217 R higher than the LD_{50} for controls. If this LD_{50} increase were due exclusively to CFU's having a D_{37} of ~ 90 R, a 15-fold increase in the size of the CFU population at the time of irradiation is predicted. An increase of this magnitude was observed in the spleen, but the spleen CFU content was not representative of the femoral marrow where only a 70% increase of CFU's was observed. Accepting the splenic and femoral CFU increments, as such, and assuming that femoral marrow contains ~ 5-10% of the total-body CFU content, and the spleen contains ~ 5%, the total-body increase would be ~ 3-fold. Therefore, unless one assumes that the spleen of the endotoxin-treated animal is the critical hematopoietic site, which is rather unlikely since splenectomized rats are protected by endotoxin (4), the increase in the stem cell population predicted by the LD_{50} shift is not realized. Since a 3-fold increase in stem cell content would account for ~ 100 R of the 217 R increase in LD_{50}, the remaining fraction of the increase would have to be attributed to other effects of endotoxin. These might include an increased rate of production of differentiated cells in the hematopoietic system, increased reticulo-

endothelia activity, and increased resistance to infection, all of which could influence the animals' survival in the critical post-irradiation period.

In certain respects such as route of injection, number of injection, the numbers of "endogenous" CFU's in the spleen do qualitatively predict the degree of endotoxin-protection. However, the lack of a consistant qualitative or quantitative correlation between the numbers of CFU's and the degree of endotoxin protection is not surprising in view of the complexity of the system. Endotoxins produce a myriad of physiologic responses, and the present data show that many factors probably influence the number of endogenous spleen nodules which are observed in endotoxin-treated animals. Therefore, the present findings may relate more to the complexity of the system than to the relationship between the CFU's and radiation sensitivity of the animal. On the other hand, the colony-forming unit may be several times removed from the "stem cell" and may not be representative of the cell population which determines radiosensitivity of the animal. Further studies are in progress which may contribute more to an understanding of the relationship between CFU's, radiation sensitivity of the animals, and radiation-protectants.

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TABLE 1

THE EFFECT OF AMOUNT OF ENDOTOXIN ADMINISTERED

Endotoxin Dose[a] 24 Hours Before 630 R	Number of Spleens	Nodules/Spleen (Mean)	S.E.M.[b]
2 μg	11	8.4	1.92
6 μg	12	9.6	1.54
12 μg	11	16.2	2.50
25 μg	17	> 40.0[c]	--
50 μg	12	33.0	2.13

[a]Administered intravenously.

[b]Standard error of the mean.

[c]Eleven spleens > 40 nodules, remainder 32, 30, 29, 29, 27, 22 nodules per spleen.

TABLE 2

THE EFFECT OF MULTIPLE ENDOTOXIN INJECTIONS

Time(s) of Endotoxin Administration[a]	Number of Spleens	Nodules/Spleen (Mean)	S.E.M.[b]
24 Hours Before 700 R	15	14.9	0.96
8 Days and 24 Hours Before 700 R	14	7.6	1.14
14 Days and 24 Hours Before 700 R	15	9.2	1.41

[a] 50 μg PIROMEN given intravenously.

[b] Standard error of the mean.

TABLE 3

THE EFFECT OF INTRAVENOUS ENDOTOXIN (50 μg)

ON THE $LD_{50/30}$ LAF_1 MICE

Treatment	$LD_{50/30}$	Number of Mice
Controls	707 (691-723)[a]	130
Endotoxin 24 Hours Before Exposure	924 (915-936)	79

[a] 95% confidence intervals are shown in parenthesis.

TABLE 4

THE EFFECT OF ENDOTOXIN ON CELLULARITY AND COLONY FORMING UNITS CONTENT OF FEMORAL BONE MARROW

	Number of Femurs	Nucleated Cells Per Femur	CFU/10^5 Nucleated Cells	Total CFU Per Femur
24 Hours After Endotoxin[a]	4	1.38×10^7	56.0	7728
	2	1.45×10^7	60.0	8700
	2	1.56×10^7	53.5	8346
	Mean	1.44×10^7	56.2	8093
Controls	4	1.10×10^7	28.0	3080
	4	1.90×10^7	33.0	6270
	2	1.70×10^7	44.0	7480
	4	1.50×10^7	26.0	3900
	Mean	1.53×10^7	31.1	4750

[a] 50 µg PIROMEN given intravenously.

TABLE 5

THE EFFECT OF ENDOTOXIN ON SIZE OF REGENERATING SPLEEN NODULES

Controls		Endotoxin Treated[b]	
Dose (R)	Mean Diameter[a]	Dose (R)	Mean Diameter[a]
425	7.3 (6.8-7.8)	700	8.7 (8.1-9.2)
		700	7.7 (7.2-8.1)
465	5.6 (5.2-5.9)	725	7.4 (6.9-7.8)
		745	8.5 (8.0-9.0)
500	6.9 (6.4-7.5)	765	8.0 (7.5-8.5)
		800	6.7 (5.7-7.8)
510	6.6 (6.0-7.2)	800	6.7 (6.0-7.4)
575	6.5 (5.6-7.3)	875	7.6 (6.7-8.5)
	$\Sigma \bar{V}$ 6.6		$\Sigma \bar{V}$ 7.7

[a]Units used: 8.0 units = 1.0 mm; parentheses include the 95% confidence limits.

[b]50 µg PIROMEN given intravenously 24 hours before radiation.

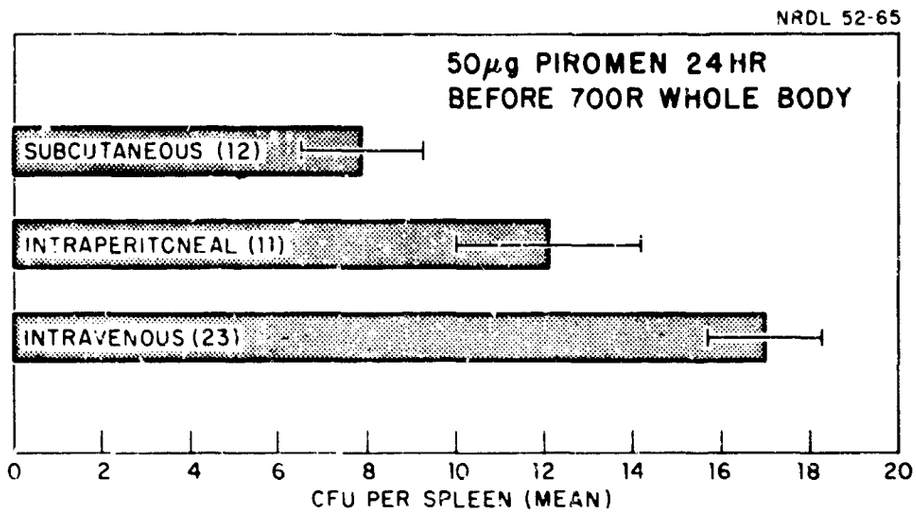

Fig. 1. Effect of route of administration on endogenous spleen colony-forming units.

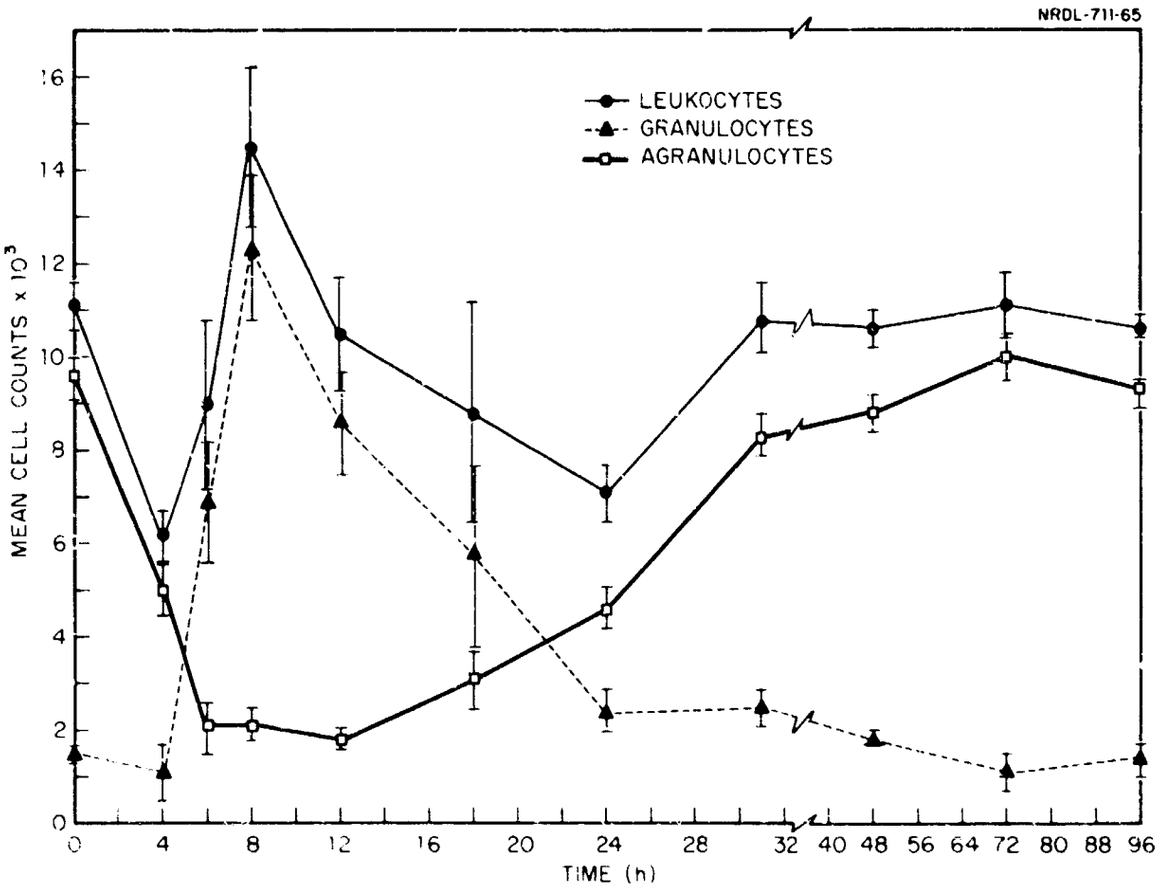

Fig. 2. Peripheral leukocyte counts following intravenous injection injection of 50 micrograms of endotoxin.

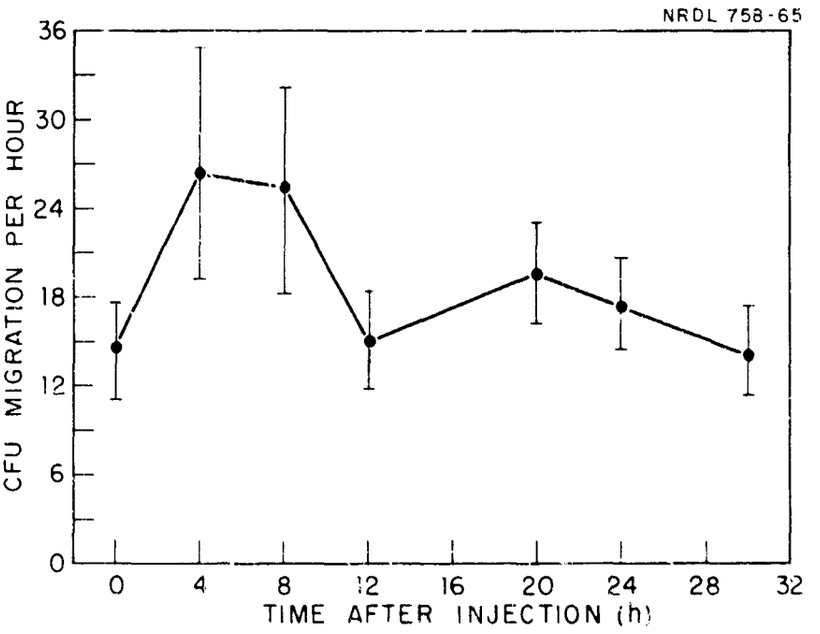

Fig. 3. Effect of endotoxin on migration of CFU's from a shielded femur to the spleen.

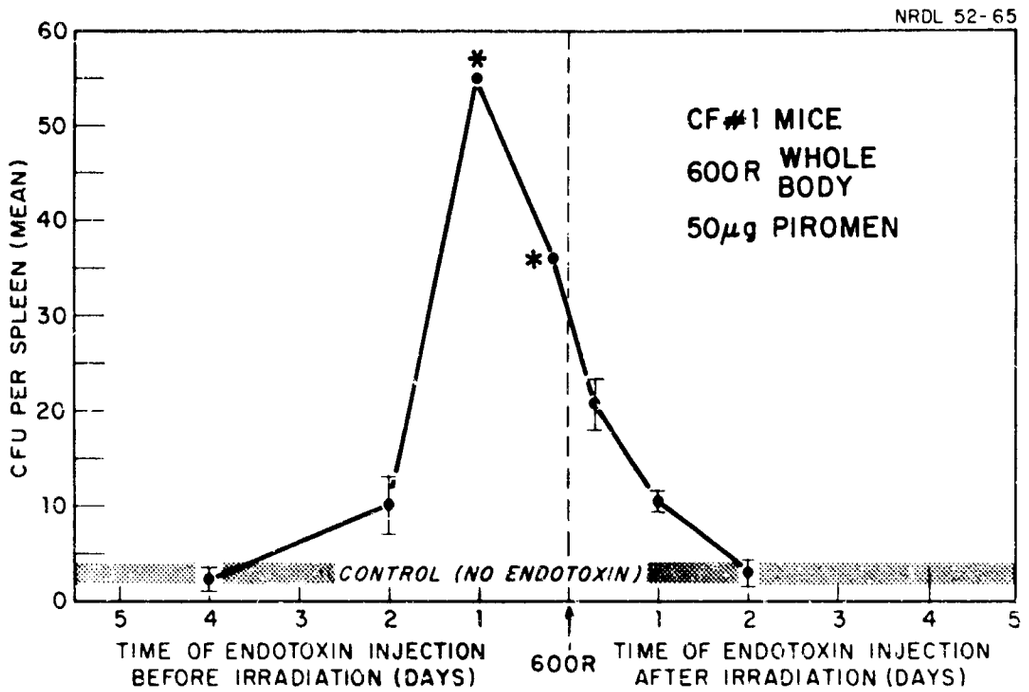

Fig. 4A. Effect of time of endotoxin injection of endogenous spleen CFU's. CF#1 mice exposed to 600 R.

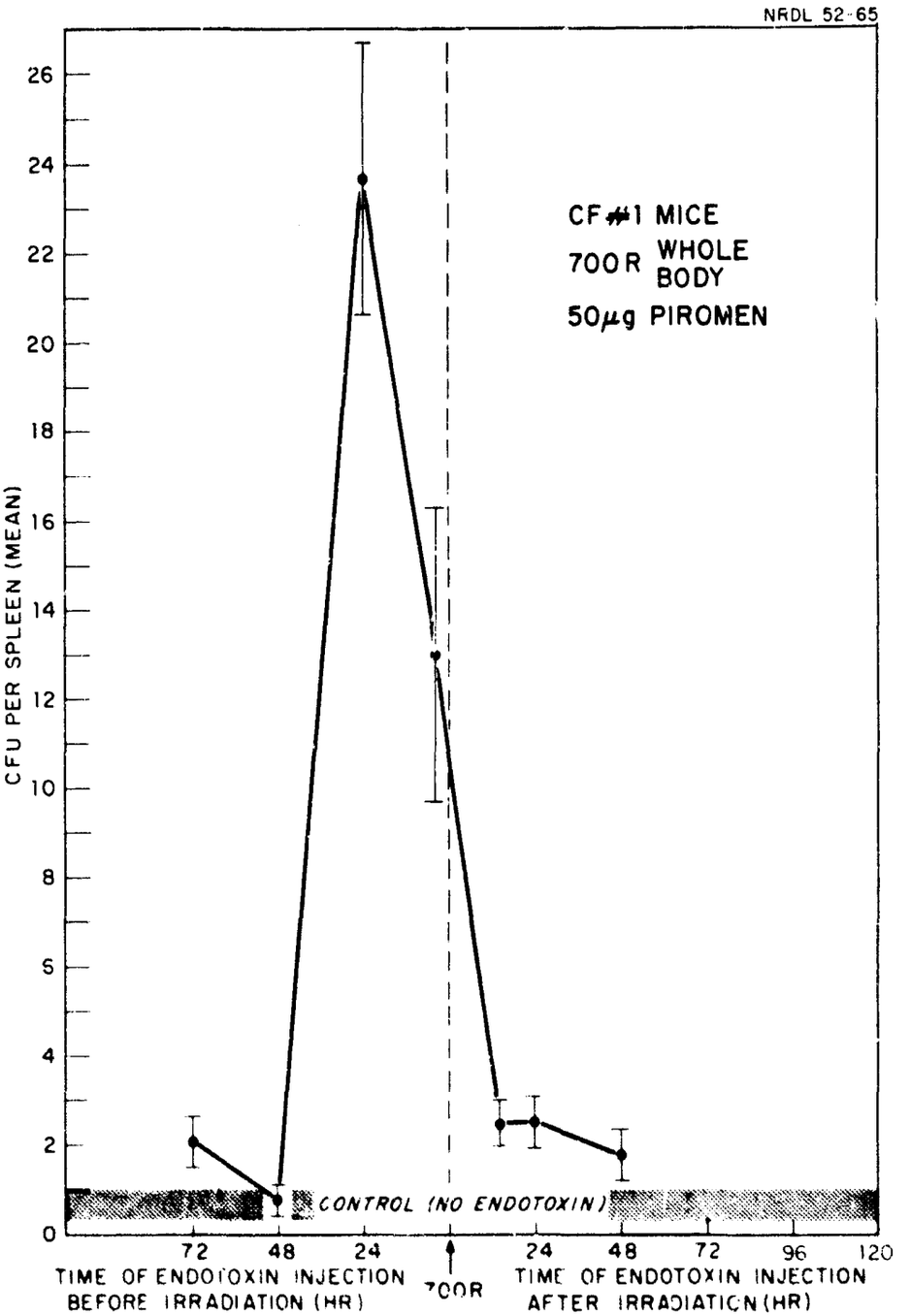

Fig. 4B. Effect of time of endotoxin injection on endogenous spleen CFU's. CF#1 mice exposed to 700 R.

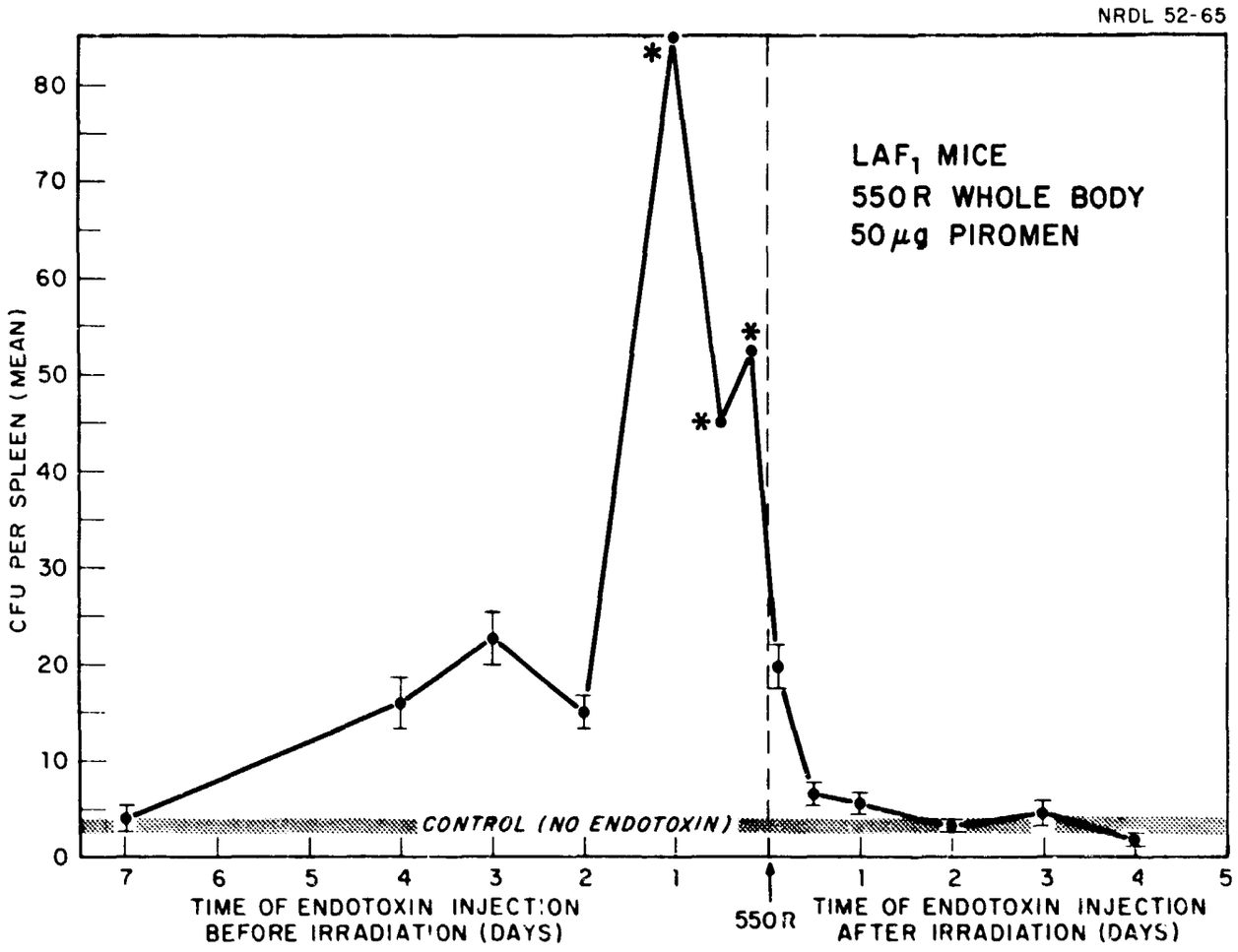

Fig. 4C. Effect of time of endotoxin injection on endogenous spleen CFU's. LAF_1 mice exposed to 550 R.

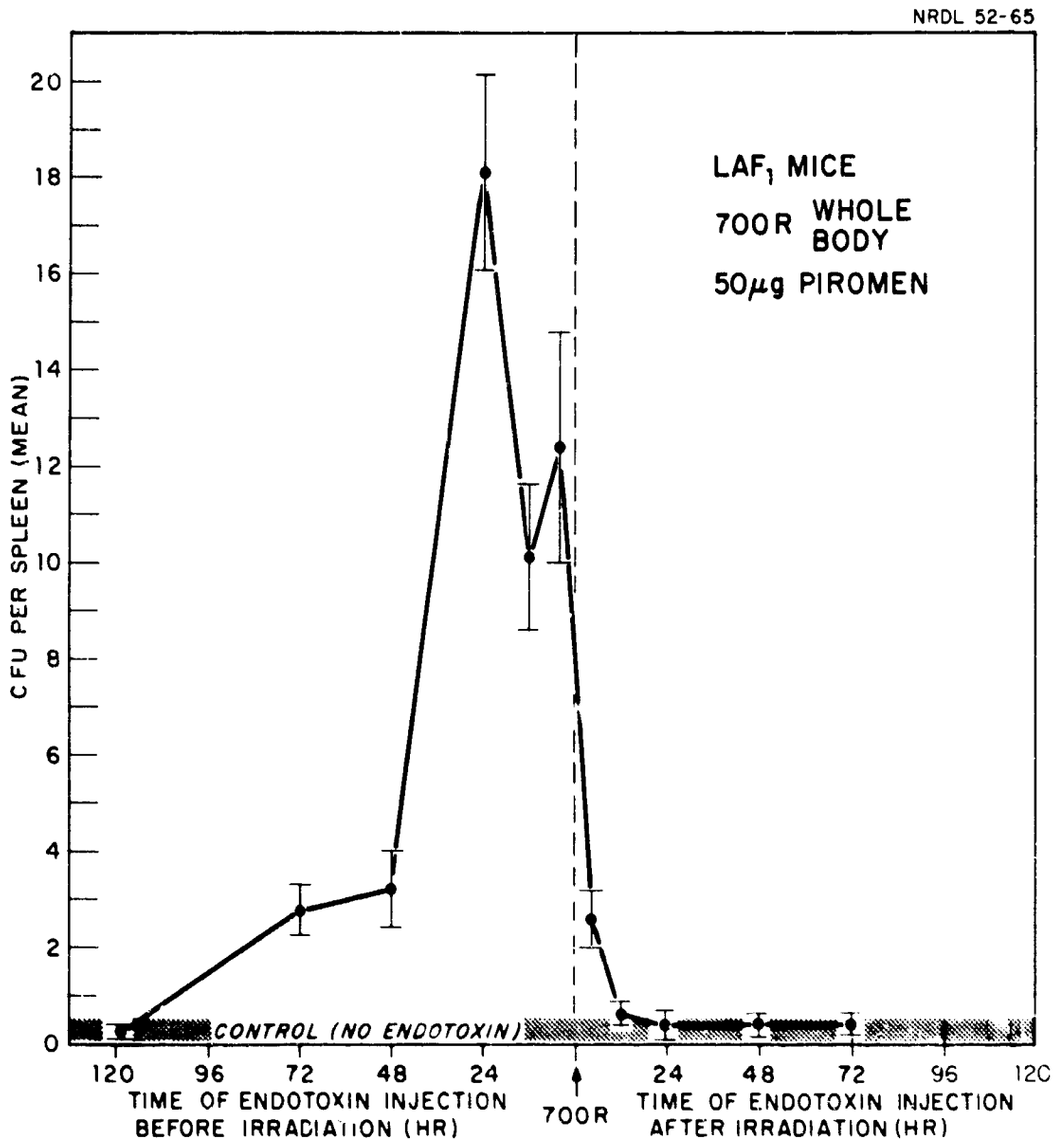

Fig. 4D. Effect of time of endotoxin injection on endogenous spleen CFU's. LAF_1 mice exposed to 700 R.

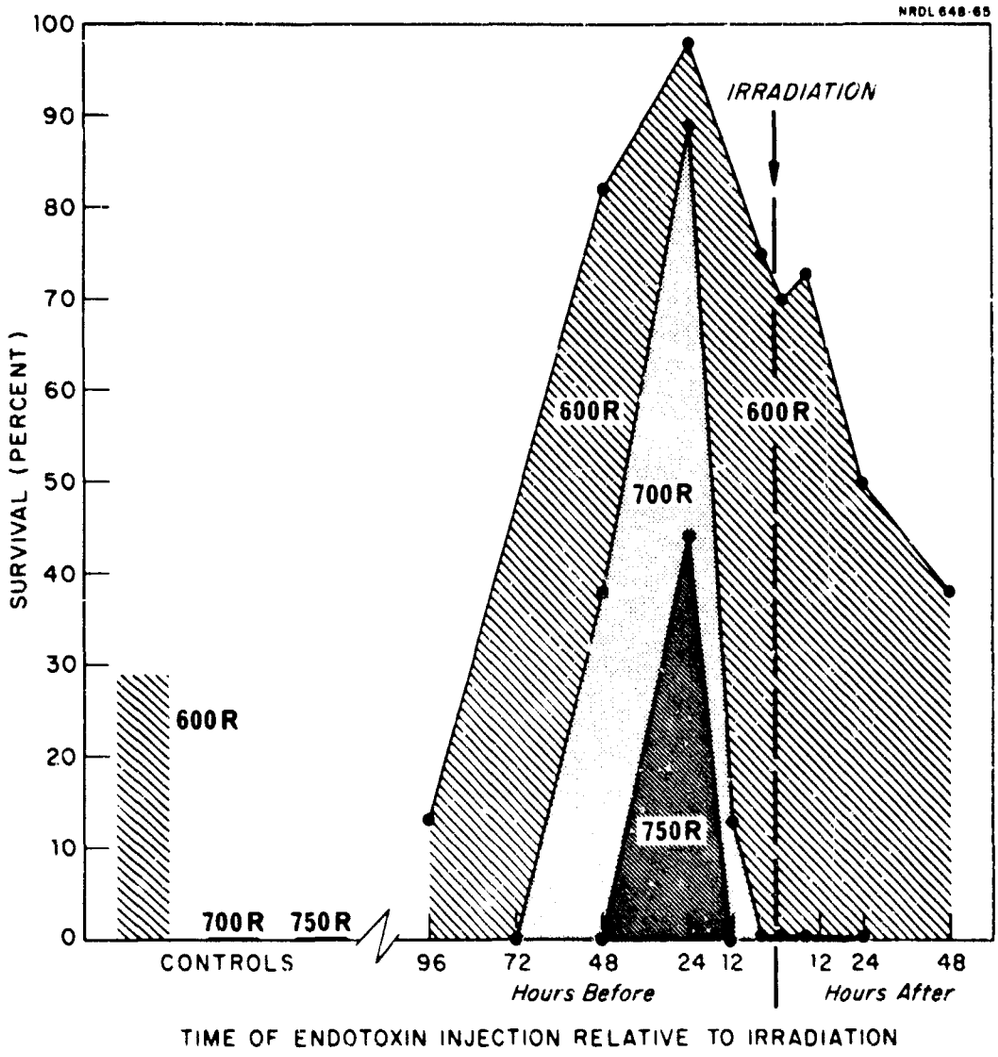

Fig. 5. The effect of time of endotoxin injection on survival of BUB mice exposed to 600, 700, or 750 R.

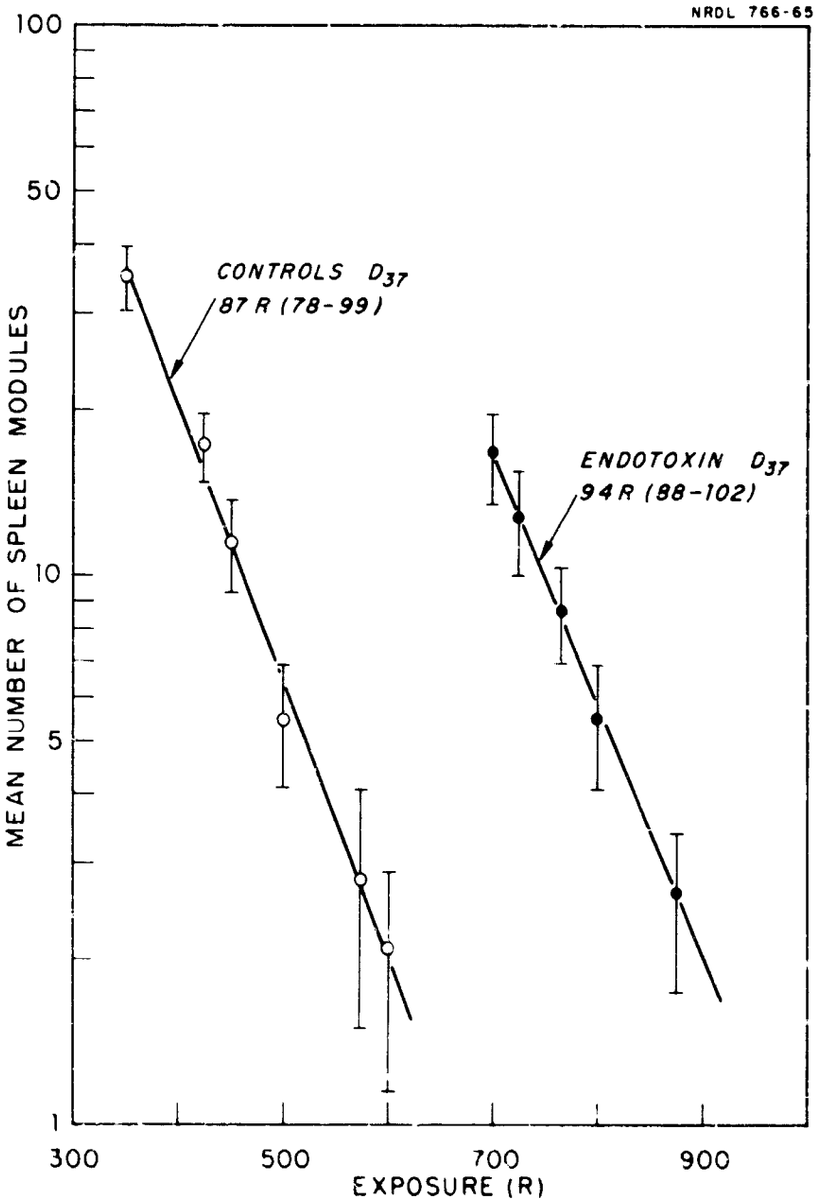

Fig. 6. Survival curves for CFU's in endotoxin-treated and normal mice.

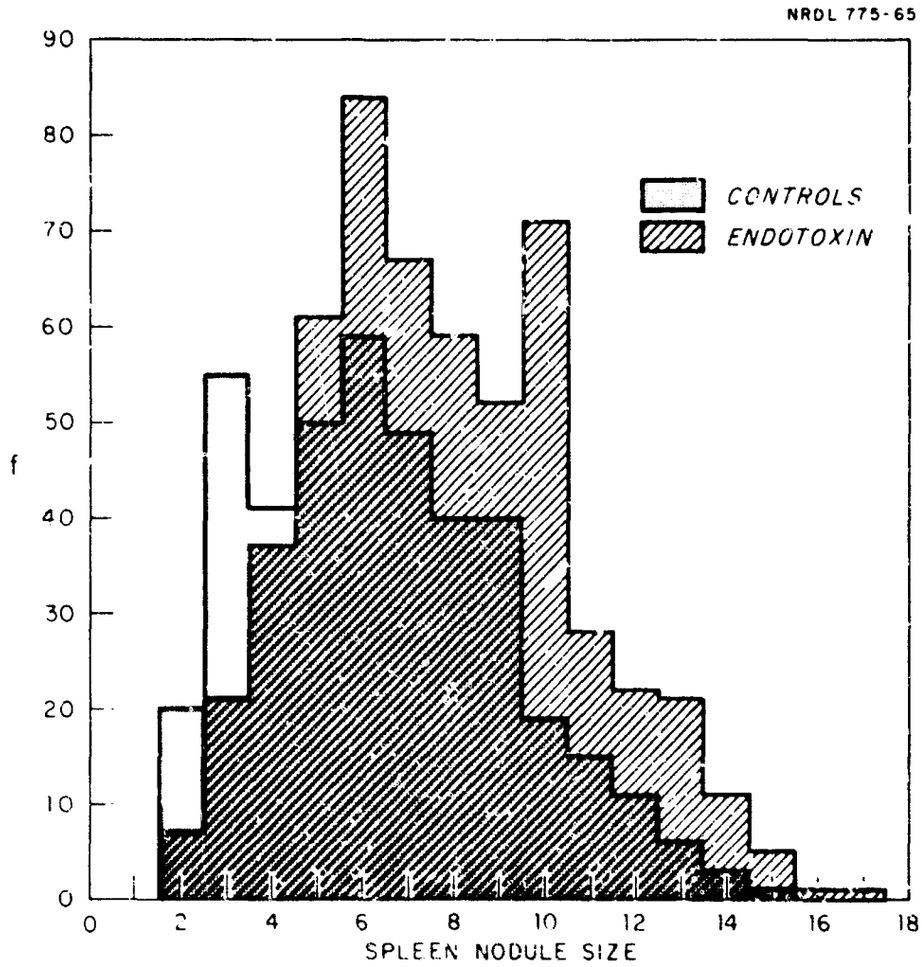

Fig. 7. Frequency distribution of nodule sizes in endotoxin-treated and control mice.

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13. ABSTRACT Earlier studies have shown that bacterial endotoxins increase survival of irradiated animals. Although these substances do not confer as much protection as do the classical chemical protectants, endotoxins do significantly increase survival when given either before or for a short time following irradiation. The mechanisms of endotoxin protection have not been clearly established, but earlier studies have shown that hematopoietic stimulation is involved. The present studies were designed to evaluate the effects of endotoxin on proliferative cells in the hematopoietic system. Methods have recently been devised to measure the numbers of certain proliferative cells within the bone marrow, spleen, and other hematopoietic sites. These proliferative cells are called colony-forming units (CFU's), and their numbers are estimated by counting the "colonies" or nodules in the spleens of irradiated mice which arise either "spontaneously" or after injection of hematopoietic cells. The present data indicate that endotoxin does not alter the radiosensitivity of endogenous splenic CFU's; the D_{37} for CFU's is ~ 90 R in both endotoxin-treated and control mice. When endotoxin is injected at the time which produces optimal survaval, the femur content of CFU's increases ~ 2 fold; whereas, the spleen content of CFU's is increased ~ 20 fold. The increased rate of CFU's migration from the femur to the spleen in endotoxin-treated animals may contribute to the relatively greater increase in splenic CFU's. The diameter of the CFU's in the endotoxin-treated mice is somewhat larger than in controls which may have implications relating to rates of cell division. Other data are presented which relate to the extent to which time of endotoxin injection and numbers of splenic CFU's can be correlated with survival of irradiated mice.

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